

Mediator coordinates PIC assembly with recruitment of CHD1.

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Authors:	Justin J Lin, Lynn W Lehmann, Giancarlo Bonora, Rupa Sridharan, Ajay A Vashisht, Nancy Tran, Kathrin Plath, James A Wohlschlegel, Michael Carey
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Public Summary:

Murine Chd1 (chromodomain helicase DNA-binding protein 1), a chromodomain-containing chromatin remodeling protein, is necessary for embryonic stem (ES) cell pluripotency. Chd1 binds to nucleosomes trimethylated at histone 3 Lys 4 (H3K4me3) near the beginning of active genes but not to bivalent domains also containing H3K27me3. To address the mechanism of this specificity, we reproduced H3K4me3- and CHD1-stimulated gene activation in HeLa extracts. Multidimensional protein identification technology (MuDPIT) and immunoblot analyses of purified preinitiation complexes (PICs) revealed the recruitment of CHD1 to naive chromatin but enhancement on H3K4me3 chromatin. Studies in depleted extracts showed that the Mediator coactivator complex, which controls PIC assembly, is also necessary for CHD1 recruitment. MuDPIT analyses of CHD1-associated proteins support the recruitment data and reveal numerous components of the PIC, including Mediator. In vivo, CHD1 and Mediator are recruited to an inducible gene, and genome-wide binding of the two proteins correlates well with active gene transcription in mouse ES cells. Finally, coimmunoprecipitation of CHD1 and Mediator from cell extracts can be ablated by shRNA knockdown of a specific Mediator subunit. Our data support a model in which the Mediator coordinates PIC assembly along with the recruitment of CHD1. The combined action of the PIC and H3K4me3 provides specificity in targeting CHD1 to active genes.

Scientific Abstract:

Murine Chd1 (chromodomain helicase DNA-binding protein 1), a chromodomain-containing chromatin remodeling protein, is necessary for embryonic stem (ES) cell pluripotency. Chd1 binds to nucleosomes trimethylated at histone 3 Lys 4 (H3K4me3) near the beginning of active genes but not to bivalent domains also containing H3K27me3. To address the mechanism of this specificity, we reproduced H3K4me3- and CHD1-stimulated gene activation in HeLa extracts. Multidimensional protein identification technology (MuDPIT) and immunoblot analyses of purified preinitiation complexes (PICs) revealed the recruitment of CHD1 to naive chromatin but enhancement on H3K4me3 chromatin. Studies in depleted extracts showed that the Mediator coactivator complex, which controls PIC assembly, is also necessary for CHD1 recruitment. MuDPIT analyses of CHD1-associated proteins support the recruitment data and reveal numerous components of the PIC, including Mediator. In vivo, CHD1 and Mediator are recruited to an inducible gene, and genome-wide binding of the two proteins correlates well with active gene transcription in mouse ES cells. Finally, coimmunoprecipitation of CHD1 and Mediator from cell extracts can be ablated by shRNA knockdown of a specific Mediator subunit. Our data support a model in which the Mediator coordinates PIC assembly along with the recruitment of CHD1. The combined action of the PIC and H3K4me3 provides specificity in targeting CHD1 to active genes.

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